Environmental Health Perspectives
Vol. 57, pp. 289-291, 1984

Perturbation of Calcium Homeostasis by CCl_4 in Rats Pretreated with Chlordecone and Phenobarbital

by Arvind K. Agarwal* and Harihara M. Mehendale*†

Male Sprague-Dawley rats were maintained on normal powdered diet or on the same diet containing 10 ppm chlordecone (CD) or 225 ppm phenobarbital (PB) for 15 days. On day 15, they received a single IP injection of a subtoxic dose of CCl_4. Induction of cytochrome P-450 was greater with phenobarbital treatment than with chlordecone, but the CCl_4-induced destruction of P-450 was similar in both groups and was progressive with the dose of CCl_4 and with time after CCl_4 administration. CCl_4 given to animals on normal diet in a dose range of 25 to 200 μL/kg did not significantly alter the P-450 levels. These findings are consistent with greater bioactivation of CCl_4 after the above two pretreatments. There was a massive accumulation of Ca^{2+} in CD- and PB-pretreated animals after CCl_4 administration, CD being more effective in this regard. Elevation of cytosolic Ca^{2+} was progressive despite the mitochondrial and microsomal sequestration of cytosolic Ca^{2+} at elevated levels. This perturbation of hepatocellular Ca^{2+} homeostasis which occurs 3 to 6 hr after CCl_4 may prevent hepatocellular repair and renovation in CD-treated animals, leading to progressive hepatic lesion, hepatic failure and animal death by 36 to 48 hr at nontoxic doses of CCl_4. Neither CD nor PB nor CCl_4 alone affected hepatic Ca^{2+}. These findings suggest that excessive Ca^{2+} accumulation may be related to the progression of hepatotoxic response to CCl_4 in CD-treated animals.

Introduction

Various chemical toxins that initiate toxic events leading to liver cell death exhibit marked alterations in intracellular Ca^{2+} homeostasis with excessive accumulation of Ca^{2+} (*1,2*). The intracellular Ca^{2+} sequestration has been implicated as a potential mediator of toxic events which lead to hepatic cell death (*3,4*). Previous work from this laboratory has established the remarkable potentiation of CCl_4 hepatotoxicity and lethality by chlordecone (chlorinated insecticide, Kepone, CD) pretreatment in male (*5,6*) and female rats (*7*). Although an enhanced bioactivation of CCl_4 in CD-pretreated rats was reported (*8*), the quantum of increased bioactivation was considered insufficient to explain the 70-fold increase in lethality in these animals as compared to phenobarbital pretreated rats which exhibited only 2-fold increase in lethality (*6*).

With this background, the changes in hepatocellular Ca^{2+} homeostasis associated with potentiation of CCl_4 toxicity by CD were investigated. Also, in view of the earlier findings indicating stimulated bioactivation of CCl_4 in CD-treated animals (*8*), it was important to determine if enhanced bioactivation of CCl_4 by CD pretreatment resulted in greater destruction of cytochrome P-450. PB pretreatment was used as a positive control for the potentiation of CCl_4 hepatotoxicity.

*Department of Pharmacology and Toxicology, The University of Mississippi Medical Center, Jackson, MS 39216.
†Author to whom reprint requests should be addressed.

Methods

Male Sprague-Dawley rats weighing 200 to 225 g (Charles River Breeding Laboratories, Wilmington, MA) were housed in a 12-hr photoperiod on a corn cob bedding untreated with any known inducers. The animals were maintained on normal commercial powdered rat chow (Ralston Purina Rat Chow Co., St. Louis, MO) or the diet containing 10 ppm CD or 225 ppm PB prepared as described previously (*5*) for 15 days. On day 15 a group of rats received a single IP injection of 100 μL CCl_4/kg in corn oil vehicle (1 mL/kg) and sacrificed at 0, 0.5, 2, 6, 12, 24 and 36 hr. Hepatic microsomal cytochrome P-450 was determined by the method of Omura and Sato (*9*). Other groups of rats received a single IP injection of 25 to 200 μL CCl_4/kg and sacrificed 12 hr later. Control animals received only the vehicle. Ca^{2+} levels in the whole liver, mitochondria, microsomes and cytosolic fraction were determined in nitric acid-digested samples by using atomic absorption spectrophotometry.

Results and Discussion

Hepatic microsomal cytochrome P-450 levels were determined at the time the animals would have received CCl_4 or at various time points after CCl_4 administration. CD treatment increased the hepatic microsomal P-450 by about 60%, whereas PB almost doubled P-450 levels (Fig. 1). CCl_4 administration (100 μL/kg) to these

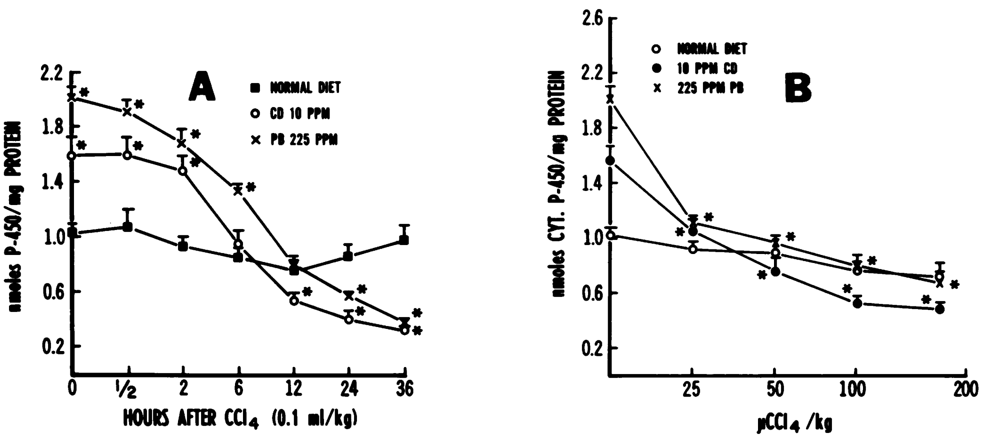

FIGURE 1. Destruction of cytochrome P-450. Male Sprague-Dawley rats were maintained on a normal powdered diet or on a diet containing 10 ppm chlordecone or 225 ppm phenobarbital for 15 days. On day 15 (*A*) they received a single IP injection of 100 µL CCl_4/kg and were sacrificed at different time points as indicated or (*B*) they received single IP injection of different doses of CCl_4 (25–200 µL/kg) and sacrificed 12 hr later. Controls received only the corn oil vehicle (1 mL/kg). Microsomal cytochrome P-450 was determined in the liver and expressed as nmoles cytochrome P-450/mg protein. Asterisks denote that the values are significantly different from zero point level of rats fed normal diet, $p < 0.05$.

rats caused a progressive and time-dependent destruction of P-450 (Fig. 1*A*). The percent destruction remained the same in both CD- and PB-pretreated animals, despite the unequal induction of cytochrome P-450. Administration of different doses of CCl_4 (25–200 µL/kg) caused a significant destruction of P-450 at all the doses (Fig. 1*B*). In the rats maintained on normal diet, these doses of CCl_4 did not affect P-450 levels. These data are suggestive of enhanced bioactivation of CCl_4 in CD and PB pretreated animals. Previous studies (*8,10*) have shown greater *in vivo* and *in vitro* metabolism of CCl_4. Since this enhanced metabolism of CCl_4 occurs at lesser increases in P-450 levels, these findings are consistent with induction of specific form(s) of CCl_4-bioactivating hemoprotein by CD (*10*). However, in view of remarkable differences in the potentiation of CCl_4 toxicity between PB and CD treatments, it is necessary to consider factors other than just bioactivation that might be playing a role in initiating or promoting hepatic cell death due to CCl_4 poisoning.

Dietary exposure to CD or PB did not influence whole liver or subcellular Ca^{2+} levels. CCl_4 administration at a dose of 200 µL/kg to rats maintained on normal diet caused a significant rise in Ca^{2+} levels, but lower doses had no effect. Previous studies suggest that these animals recover to normal by 36 hr (*11,12*). A significant elevation in whole liver Ca^{2+} levels was evident after CCl_4 administration to both CD- and PB-pretreated rats at all four doses used (Fig. 2*A*), but the increase was much higher in CD-pretreated animals. These results are consistent with our earlier observations which indicated that animals receiving CD + 100 µL CCl_4/kg exhibit total hepatic failure with extensive hepatocellular necrosis which progresses and leads to animal death by 36 hr. In contrast, the animals receiving normal diet or PB + CCl_4 do not show such extensive necrosis, and these animals recover later (*6,10,12*).

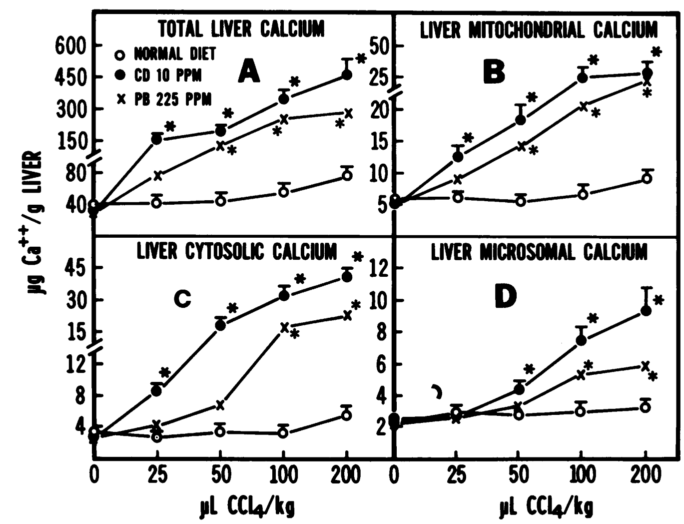

FIGURE 2. Ca^{2+} levels in subcellular fractions of the liver. Animals were maintained and treated as described in Fig. 1*B*. Ca^{2+} levels in (*A*) whole liver, (*B*) mitochondria, (*C*) cytosol and (*D*) microsomes were determined. The values were expressed as µg Ca^{2+}/g liver. Asterisks denote that the values are significantly different from zero point level of rats fed normal diet, $p < 0.05$.

Increased Ca^{2+} levels after CCl_4 administration were readily evident in mitochondria (Fig. 2*B*) due to a continuous influx of extracellular Ca^{2+} in cytosol (Fig. 2*C*). Microsomes also play a role in sequestering increased cytosolic Ca^{2+} levels (Fig. 2*D*); this was especially evident at higher doses of CCl_4. Plasma membrane changes taking place presumably due to increased lipid peroxidation or other factors consequent to CCl_4 bioactivation disrupt the permeability barrier with a consequent influx of Ca^{2+} which results in massive Ca^{2+} accumulation in the cell. Although, hepatic mitochondria and microsomes continue to regulate ever increasing cytosolic Ca^{2+} by increased sequestration, the cytosolic Ca^{2+} levels still remain high (Fig. 2*C*), leading finally to cell death. Our earlier time-course histomorphometric studies (*11,12*) indicate that whereas animals treated with CCl_4 (100 μL/kg) recover from liver damage by virtue of hepatocellular repair and renovation, those treated with CD + CCl_4 do not. Instead, 3–4 hr after CCl_4 when hepatocellular repair would have occurred (*11,12*), a progressive increase in cytosolic Ca^{2+} occurs in animals receiving the CD + CCl_4 combination treatment, suggesting a cause-effect relationship. In animals receiving CCl_4 alone, Ca^{2+} homeostasis is unperturbed, allowing the hepatocellular repair, renovation and recovery.

REFERENCES

1. Agarwal, A. K., and Mehendale, H. M. Excessive hepatic accumulation of intracellular Ca^{++} in chlordecone potentiated CCl_4 toxicity. Toxicology 30: 17–24 (1984).
2. Farber, J. L. Reactions of the liver to injury: necrosis. In: Toxic Injury of the Liver, Part A (E. Farber and M. M. Fisher, Eds.), Marcel Dekker, New York, 1979, pp. 215–241.
3. Schanne, F. A. X., Kane, A. B., Young, E. E., and Farber, J. L. Calcium dependence of toxic cell death: a final common pathway. Science 206: 700–702 (1979).
4. Smith, M. I., Thor, H., and Orrenius, S. Toxic injury to isolated hepatocytes is not dependent on extracellular calcium. Science 213: 1257–1259 (1981).
5. Curtis, L. R., Williams, W. L., and Mehendale, H. M. Potentiation of carbon tetrachloride following preexposure to chlordecone (Kepone) in the male rat. Toxicol. Appl. Pharmacol. 51: 283–293 (1979).
6. Klingensmith, J. S., and Mehendale, H. M. Potentiation of CCl_4 lethality by chlordecone. Toxicology Letters 11: 149–154 (1982).
7. Agarwal, A. K., and Mehendale, H. M. Potentiation of CCl_4 hepatotoxicity and lethality by chlordecone in female rats. Toxicology 26: 231–242 (1983).
8. Klingensmith, J. S., and Mehendale, H. M. Hepatic microsomal metabolism of CCl_4 after pretreatment with chlordecone, mirex or phenobarbital in male rats. Drug Metab. Dispos. 11: 329–334 (1983).
9. Omura, T., and Sato, R. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J. Biol. Chem. 239: 2370–2378 (1964).
10. Mehendale, H. M. Potentiation of halomethane hepatotoxicity. Fundam. Appl. Toxicol. 4: 295–308 (1984).
11. Lockard, V. G., Mehendale, H. M., and O'Neal, R. M. Chlordecone induced potentiation of carbon tetrachloride hepatotoxicity. A morphometric and biochemical study. Exptl. Mol. Pathol. 39: 246–255 (1983).
12. Lockard, V. G., Mehendale, H. M., and O'Neal, R. M. Chlordecone induced potentiation of carbon tetrachloride hepatotoxicity. A light and electron microscopic study. Exp. Molec. Pathol. 39: 230–245 (1983).